EFFECTS OF SODIUM, POTASSIUM, AND CALCIUM IONS ON THE ISOLATED HEART OF THE MEALWORM, TENEBRIO MOLITOR L[1]

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Insect tissue has great plasticity which enables it to maintain its activities in very abnormal media. The purpose of this investigation is to determine the tolerance of insects to different concentrations of sodium, potassium and calcium, as well as to changes in the osmotic pressure by the use of isolated heart preparations as indicators. Since no work is available on changes during metamorphosis of ion concentrations or tolerance to compositions of saline as it changes during metamorphosis these experiments were performed on a holometabolous insect, *Tenebrio molitor,* during the larval, pupal, and adult stages.

MATERIAL AND METHODS

The method for the preparation of glassware was that of Gese (1950) with the exception that no permanganate bath was used. Larvae of *Tenebrio molitor* were grown in chick growing mash dampened several times a week. Prepupae were collected daily and kept in beakers at 30°C. Each day, pupae were removed, placed in dated beakers and stored at 30° C. In this way the age of each pupa, within 24 hours, was obtained. Three-day pupae were used because at 30°C. this stage is the mid-point of the pupal period. Young adults from two to three weeks after emergence were used.

An analysis was made of the ion concentration and osmotic pressure of the blood of larvae, pupae, and adults. Blood was obtained from the larva and adult by removing a metathoracic leg with sharp scissors at the junction of the coxa and thorax. Blood was obtained from the pupa by cutting the tip of the abdomen with sharp scissors. In all cases, it was allowed to

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drip into a porcelain spot-plate, while pressure was exerted on the abdomen. Each analysis was made on 0.5 ml. of pooled blood using the Beckman flame photometer. For each stage, six determinations were made for ion concentration.

The osmotic pressures of the blood during larval, pupal, and adult stages were determined by Barger's vapor pressure method as used by Ludwig (1951). The molality and the osmotic pressures were calculated from the NaCl equivalence.

The effects of various solutions on the heart beats were determined by the following procedure. The hearts were prepared by the method of Yeager and Hager (1934). Since they were not clearly visible, several drops of 0.1 per cent neutral red were allowed to remain on the preparation for 30 seconds and then drained off. When the preparations were kept for 24 hours, neutral red was again added for better visibility. Aerated solutions at 25°C. were allowed to flow over the hearts. The apparatus used was a modification of that devised by Yeager and Hager (1934). A binocular dissecting microscope was used for observation and the time for ten beats was measured at 5 minute intervals over a three-hour period. The hearts which were beating at the end of this time were kept for 24 hours during which time the solution was allowed to drip more slowly on the preparation. For each solution tested, at least nine heart preparations were used. The solution was evaluated by the ability of the immersed hearts to beat at a uniform rate over a 3-hour and a 24-hour period.

The effects of osmotic pressure on the heart of the three stages were determined by taking a favorable isotonic solution and varying its osmotic pressure. Hypotonic solutions were prepared by diluting the isotonic, and hypertonic by adding a determined amount of the proper mixture of chloride salts.

OBSERVATIONS

The concentration of sodium, potassium, and calcium ions, as well as the osmotic pressure of the blood of the three stages are listed in Table I. The average concentration of inorganic cations in the larva, expressed as milligrams per cent, are sodium 177.7, potassium 128.3, and calcium 22.3; in the pupa they are 41.3, 74.0 and 7.8; and in the adult 83.5, 92.3, and 27.1, respectively.

The larval blood is isotonic to 0.37 molal NaCl (or KCl), and 0.26 molal $CaCl_2$; pupal blood to 0.40 molal NaCl and 0.28 molal $CaCl_2$; and adult blood to 0.2 molal NaCl, and 0.14 molal $CaCl_2$.

In isotonic NaCl the hearts beat at a moderate rate and the beating of the larval heart persisted for 44, the pupal heart for 53, and the adult heart for 32 minutes. Corresponding values for $CaCl_2$ were 38, 32, and 17 minutes, respectively. None of the

TABLE I

Na, K, and Ca Content and Osmotic Pressure of the Blood.

Determination	Larva	Pupa	Adult
	Composition		
	mg. per cent	mg. per cent	mg. per cent
Sodium			
Maximum	202.3	47.6	86.3
Minimum	142.4	38.2	80.1
Average	177.7	41.3	83.5
Potassium			
Maximum	146.7	86.1	97.4
Minimum	102.5	58.7	90.1
Average	128.3	74.0	92.3
Calcium			
Maximum	26.3	8.8	30.4
Minimum	18.4	6.5	25.0
Average	22.3	7.8	27.1
	Osmotic Pressure		
NaCl equivalent, in per cent	2.14 (obs.)	2.40 (obs.)	1.45 (obs.)
Molality	0.37 (calc.)	0.40 (calc.)	0.20 (calc.)
Atmospheres	17.04 (calc.)	19.20 (calc.)	11.64 (calc.)

hearts beat in isotonic solutions of potassium chloride. The pupal heart beat in distilled water for 12 minutes. For each stage, sodium was found to be the least toxic, followed by calcium and then by potassium.

Table II shows the various ion ratios needed for maintaining the heart beat of the larva. The Na/K ratio could be varied from 1.4 to 22.4 without any appreciable toxic effects. When the ratio of K/Ca was 0.33 to 3, the resulting physiological solutions were excellent. However, when the potassium was increased so that the ratio was 4, irregularities were seen in the heart and the beating did not continue for 24 hours. Very slight irregulari-

TABLE II

MILLILITERS OF ISOTONIC SOLUTIONS AND ION RATIOS NEEDED FOR MAINTAINING THE HEART BEAT OF THE LARVA.

NaCl	KCl	$CaCl_2$	Ion ratios		Beats/min.	Period of activity (Hours)
			Na/K	K/Ca		
....	100	24	0.00	1.00	18.5	3
256	100	24	1.40	1.00	12.5	24
512	100	24	2.80	1.00	17.8	24
1024	100	24	5.60	1.00	19.3	24
2048	100	24	11.20	1.00	18.0	24
4096	100	24	22.40	1.00	25.3	24
5120	100	24	28.00	1.00	34.6	3
256	100	12	1.40	2.00	22.9	24
256	100	9	1.40	3.00	19.5	24
256	100	6	1.40	4.00	18.7	3
256	100	48	1.40	0.50	17.7	24
256	100	72	1.40	0.33	16.9	24
256	100	96	1.40	0.25	11.5	3

ties were seen in the beats of hearts treated with solutions having a potassium to calcium ratio of 0.5 and 0.33, but the beating persisted for 24 hours. However, when this ratio was decreased to 0.25, the beats became very irregular and did not last for 24 hours.

Table III shows the various ion ratios needed for maintaining

TABLE III

MILLILITERS OF ISOTONIC SOLUTIONS AND ION RATIOS NEEDED FOR MAINTAINING THE HEART BEAT OF THE PUPA.

NaCl	KCl	$CaCl_2$	Ion ratios		Beats/min.	Period of activity (Hours)
			Na/K	K/Ca		
....	179	26	0.00	1.00	10.3	0.3
165	179	26	0.60	1.00	23.9	1.5
330	179	26	1.10	1.00	11.7	2.5
660	179	26	2.20	1.00	29.9	24.0
1320	179	26	4.40	1.00	19.7	24.0
2640	179	26	8.80	1.00	15.9	24.0
3300	179	26	11.00	1.00	14.3	3.0
660	179	13	2.20	2.00	21.5	24.0
660	179	9	2.20	3.00	27.6	24.0
660	179	6	2.20	4.00	20.5	3.0
165	45	52	2.20	0.50	10.8	3.0

the heart beat of the pupa. The heart did not beat in a solution of the same ratio as found in the pupal blood, that is, where the Na/K ratio is 0.6. When the Na/K ratio was increased to 2.2, the resulting solution was satisfactory since the irregularities in beating decreased to a minimal value, and the beating continued for 24 hours. The Na/K ratio could vary from 2.2 to 8.8 without any appreciable toxic effects. The K/Ca ratio may vary from 1 to 3 without any effect on regularity. However, when the ratio was increased to 4 or decreased to 0.5, irregularities were observed and the beating lasted only 3 hours.

Table IV shows the various ion ratios needed for maintaining

TABLE IV

MILLILITERS OF ISOTONIC SOLUTIONS AND ION RATIOS NEEDED FOR MAINTAINING THE HEART BEAT OF THE ADULT.

NaCl	KCl	$CaCl_2$	Ion ratios Na/K	Ion ratios K/Ca	Beats/min.	Period of activity (Hours)
....	111	44	0.00	1.00	24.9	3
136	111	44	0.90	1.00	24.7	24
272	111	44	1.80	1.00	43.5	24
544	111	44	3.60	1.00	55.0	24
1088	111	44	7.20	1.00	51.7	24
2176	111	44	14.40	1.00	56.6	24
2720	111	44	18.00	1.00	42.1	3
136	111	22	0.90	2.00	23.1	24
136	111	17	0.90	3.00	19.7	24
136	111	11	0.90	4.00	21.8	12
136	111	88	0.90	0.50	23.2	24
136	111	132	0.90	0.33	26.3	12

the heart beat of the adult. When the Na/K ratio was the same as found in the blood of the adult, that is 0.9, the beats were regular and lasted 24 hours. When the ratio was increased, the beating became stronger and also continued for the 24-hour period. The Na/K ratio could vary from 0.9 to 14.4 without any appreciable effect. It was found that when the K/Ca ratio ranged from 0.5 to 3, the resulting physiological solutions were excellent. However, when the potassium was increased so that the ratio is 4, slight irregularities were seen and the hearts did not beat for 24 hours. Slight irregularities were also seen in the beats of hearts treated with the solution having a K/Ca ratio of 0.5, although the beating persisted for 24 hours. However, when the ratio was decreased so that it was 0.33 or lower, the beats became irregular and did not persist for 24 hours.

Varying the osmotic pressure of the perfusion solution showed that when the osmotic pressure was decreased to below ½ its isotonic value, the larval heart beat became faint and slightly irregular but lasted for 3 hours. Hearts in an isotonic solution (17.02 atmospheres), or in one ¾ of its isotonic value (12.77 atmospheres), beat regularly for 24 hours. However, when the osmotic pressure was 1¼ or more times its isotonic value, irregularities were seen and survival time was greatly reduced. With hypotonic solutions the beats were faint and irregular and with hypertonic solutions they were vigorous although infrequent. Solutions which were hypertonic seemed to be less toxic than hypotonic solutions since the immersed hearts beat for a longer time, although the beating was faint. When the osmotic pressure was decreased to below ½ its isotonic value, the pupal heart beat became faint and slightly irregular but continued for 3 hours. Pupal hearts in an isotonic solution (19.20 atmospheres), or in one which is ¾ of its isotonic value (14.40 atmospheres), beat regularly for 24 hours. However, when the osmotic pressure was 1¼ or more times its isotonic value, irregularities were seen and survival time was greatly reduced. Solutions which are hypertonic seemed to be very toxic to the pupal heart, while those that are hypotonic seemed to be less toxic since the hearts beat for a longer time, although the beating was faint. When the osmotic pressure decreased below ¾ of the isotonic value, the heart beat of the adult did not persist for 24 hours. When the osmotic pressure of the solution was isotonic (11.64 atmospheres) or increased to 1¼ of the isotonic value (14.55 atmospheres), the solutions were good since the beats were regular and persisted for 24 hours. In solutions with the osmotic pressure 1½ its isotonic value the beating did not last the 24-hour period although the beats were strong and regular. When the osmotic pressure was increased to twice its isotonic value, the beats became irregular but lasted over 3 hours. With very hypertonic solutions the beats were vigorous, although infrequent. Hypertonic solutions, in contrast to their effects on the larval and pupal hearts, appeared to be less toxic than the hypotonic to the hearts of the adults.

DISCUSSION

The ion concentrations found in the blood of the *Tenebrio molitor* larva, expressed as milliequivalents per liter (77.3 for sodium, 32.9 for potassium), agree with 77.0 and 32.0 obtained by Ramsay (1953) for the larva of this species. The calcium concentration of 11.2 agrees with values ranging from 10.9 to 46.4 milliequivalents per liter, found in various species of Coleoptera and tabulated by Duchâteau, Florkin, and Leclercq (1953). Blood of the pupa obtained from the hemocoele is milky and contains fatty granules. Ion concentrations of pupal blood, expressed in milliequivalents per liter (18.0 for sodium, 19.0 for potassium and 3.9 for calcium), showed a marked decrease during metamorphosis from larva to pupa and a shift from a high to a low sodium content. It was thought that the marked shift in the sodium to potassium ratio might be caused by a high potassium content of the granules. Samples of blood were centrifuged to remove the granules and readings were then made on the clear hemolymph; the results showed no change in sodium and calcium but a marked increase in potassium. This experiment indicates that the high potassium content is not due to the presence of granules in the blood. There was a slight increase in sodium during metamorphosis of the pupa to the adult. Further experiments on other holometabolous insects may help in the explanation of the sodium shift.

An explanation of the fact that the pupal blood has such a high osmotic pressure as compared with the larval and adult is that in insect blood, organic material is more effective osmotically than inorganic ions (Bishop, Briggs and Ronzoni, 1925; Ludwig, 1951), and so the greater amount of organic material added to the blood during metamorphosis due to the breakdown of larval tissues may therefore account for it.

Isotonic solutions with same ion ratios as found in the blood as well as modifications were prepared and used to determine the tolerance of the insect tissue using isolated heart preparations as indicators. A solution was satisfactory when hearts immersed in it could beat for 24 hours. The most satisfactory Na/K ratio was 5.6 in the larva, but it could be varied from 1.4 to 22.4 without any appreciable toxic effects. Comparable values for the pupa were 4.4 with a range of 2.2 to 8.8; and for the adult, 7.1 and a range of 0.83 to 14.2. The most satisfactory K/Ca ratio for all three stages was 3, but it could be varied from 1 to 3. The present observations are in accord with the findings of Barsa (1954) and Ludwig, Tracey, and Burns (1957) that insect tissue is tolerant to a wide range in the ratio of Na/K, but is more sensitive to that of K/Ca. It is to be remembered that although there is a wide range in the ratios of Na/K, a difference in tolerance between stages is noticed.

Osmotic pressure is also an important factor in an insect saline solution. Drieux (1950) found that hypertonic solutions retard the frequency and increase the amplitude of heart beat of the bee moth, *Galleria mellonella,* and hypotonic solutions have an opposite effect. His results are similar to those of Barsa (1954) and those reported here. In the present experiments, hypertonic solutions were more toxic to the hearts of larvae and pupae than to those of the adults. This observation may result from the fact that the isotonic solution for both of these stages has a very high osmotic pressure. Barsa (1954) showed that, although the osmotic pressure of the blood of two insects she used (*C. viridifasciata* and *S. walkeri*) are very different, their hearts appear to be tolerant to solutions having approximately the same tonicity. It was noted, that the larval blood of *T. molitor* has an osmotic pressure of 17.02 atmospheres, and the hearts beat normally in solutions which varied from 12.77 to 17.02 atmospheres. On the other hand, the pupal blood has an osmotic pressure of 19.20 atmospheres and pupal hearts beat normally in solutions ranging from 14.40 to 19.20 atmospheres. Similar figures for the adult were 11.64 to 14.55 atmospheres. Therefore, the hearts of each stage appear to be tolerant to a solution with an osmotic pressure of approximately 14.5 atmospheres.

With this information a solution was allowed to be devised which contained 16.0 g. of NaCl, 1.4 g. of KCl, and 1.0 g. of $CaCl_2$ per liter. Although this solution was quite well tolerated by all stages it is clearly indicated that a physiological solution good for one stage might be wholly insufficient to another stage, because of difference in tolerance between different stages. Experimenters using physiological solutions should be aware of this.

SUMMARY

The blood of larvae, pupae, and adults of the mealworm was analyzed. The average concentrations of inorganic cations in the larva, expressed as milligrams per cent, are sodium 177.7, potassium 128.3, and calcium 22.3; in the pupa they are 14.3, 74.0, and 7.8, respectively; and in the adult, 83.5, 92.3, and 27.1. The osmotic pressures of the larval, pupal, and adult blood are 17.02, 19.20, and 11.64 atmospheres, respectively.

The effects of isotonic chloride solutions of sodium, potassium, calcium, and of distilled water on the heart beat of the larva, pupa, and the adult were determined. For all the stages, sodium was found to be the least toxic ion, followed by calcium and then by potassium. None of the hearts beat in isotonic solutions of potassium chloride.

The Na/K ratio for the larval heart could be varied from 1.4 to 22.4 without any appreciable toxic effects. The ratios of K/Ca necessary for maintaining the normal heart beat of the larva may vary from 0.33 to 3. The larval hearts beat normally in solutions having osmotic pressures from 12.77 to 17.02 atmospheres.

The heart of the pupa did not beat in a solution with the Na/K ratio the same as in the pupal blood (0.6). When the Na/K ratio was increased to 2.2, the resulting solution was satisfactory. The K/Ca ratio may vary from 1 to 3. The heart of the pupa beat normally in solutions having osmotic pressures from 14.4 to 19.0 atmospheres.

The Na/K ratio for the adult heart could be varied from 0.9 to 14.4. The ratio of K/Ca may vary from 1 to 3. The adult hearts beat normally in solutions having osmotic pressures of 11.64 to 14.55 atmospheres.

A solution in which the hearts of each stage will beat for at least 24 hours is composed of 16.0 g. of NaCl, 1.4 g. of KCl, and 1.0 g. of $CaCl_2$ per liter.

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